

CLAIMS

We claim:

✓ 1. A method of sequencing a plurality of target nucleic acids each comprising a first domain and a adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
a) providing a plurality of hybridization complexes each comprising a target sequence and a sequencing primer that hybridizes to the first domain of said target sequence, said hybridization complexes attached to a surface of a substrate;
b) extending each of said primers by the addition of a first nucleotide to the first detection position using a first enzyme to form an extended primer; and
c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primers.

15 2. A method according to claim 1 wherein said hybridization complexes are attached to microspheres distributed on said surface.

3. A method according to claim 1 wherein said sequencing primers are attached to said surface.

20 4. A method according to claim 1 wherein each of said hybridization complexes comprises said target sequence, said sequencing primer and a capture probe covalently attached to said surface.

25 5. A method according to claim 1 wherein each of said hybridization complexes comprises said target sequence, said sequencing primer, an adapter probe and a capture probe covalently attached to said surface.

30 6. A method according to claim 1 further comprising:
d) extending said extended primer by the addition of a second nucleotide to the second detection position using said enzyme; and
e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primers.

35 7. The method according to claim 1 wherein said PPi is detected by a method comprising:
a) contacting said PPi with a second enzyme that converts said PPi into ATP; and
b) detecting said ATP using a third enzyme.

8. A method according to claim 7 wherein said second enzyme is sulfurylase.

9. A method according to claim 7 wherein said third enzyme is luciferase.

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10. A method of sequencing a target nucleic acid comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
a) providing a hybridization complex comprising said target sequence and a capture probe covalently attached to a microsphere on a surface of a substrate; and
b) determining the identity of a plurality of bases at said target positions.

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11. A method according to claim 10 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.

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12. A method according to claim 10 wherein said sequencing primer is said capture probe.

13. A method according to claim 10 wherein said determining comprises:
a) providing a sequencing primer hybridized to said second domain;
b) extending said primer by the addition of a first nucleotide to the first detection position using a first enzyme to form an extended primer;
c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;
d) extending said primer by the addition of a second nucleotide to the second detection position using said enzyme; and
e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

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14. The method according to claim 13 wherein said PPi is detected by a method comprising:
a) contacting said PPi with a second enzyme that converts said PPi into ATP; and
b) detecting said ATP using a third enzyme.

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15. A method according to claim 14 wherein said second enzyme is sulfurylase.

16. A method according to claim 14 wherein said third enzyme is luciferase.

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17. A method according to claim 10 wherein said determining comprises:
a) providing a sequencing primer hybridized to said second domain;

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- b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;
- c) determining the identification of said first protected nucleotide;
- d) removing the protection group;
- e) adding a second protected nucleotide using said enzyme; and
- f) determining the identification of said second protected nucleotide.

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Sub B ~~18.~~ A kit for nucleic acid sequencing comprising:

- a) a composition comprising:
 - i) a substrate with a surface comprising discrete sites; and
 - ii) a population of microspheres distributed on said sites; wherein said microspheres comprise capture probes;
- b) an extension enzyme; and
- c) dNTPs.

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19. A kit according to claim 18 further comprising:

- d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and
- e) a third enzyme for the detection of ATP.

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20. A kit according to claim 18 wherein said dNTPs are labeled.

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21. A kit according to claim 20 wherein each dNTP comprises a different label.